Horizontal Transmission of Chronic Wasting Disease in Reindeer

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We challenged reindeer by the intracranial route with the agent of chronic wasting disease sourced from white-tailed deer, mule deer, or elk and tested for horizontal transmission to naive reindeer. Reindeer were susceptible to chronic wasting disease regardless of source species. Horizontal transmission occurred through direct contact or indirectly through the environment.

Reindeer are susceptible to chronic wasting disease (CWD) after experimental oral challenge (*1*), and recently, CWD was identified in a free-ranging reindeer in Norway (*2*,*3*). Horizontal transmission is the primary mode of CWD transmission in deer. Direct horizontal transmission occurs when naive animals are exposed to infectious excreta (i.e., saliva, urine, feces) during close contact with CWD-affected animals (reviewed in *4*). Indirect horizontal transmission occurs through exposure to environments contaminated with infectious material (e.g., excreta or decomposed carcasses) (*5*,*6*).

The Eurasian reindeer (*Rangifer tarandus tarandus*) is closely related to the North American caribou (*R. t. caribou*, *R. t. granti*, *R. t. groenlandicus*). In North America, overlapping geographic ranges of free-ranging populations of potentially CWD-infected white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), or elk (*Cervus elaphus nelsoni*) present a risk for horizontal transmission to caribou. Exposure also could occur in farmed populations where contact occurs between reindeer and captive and/or free-ranging CWD-affected cervids. We investigated the transmission of CWD from white-tailed deer, mule deer, or elk to reindeer through the intracranial route and assessed them for direct and indirect horizontal transmission to uninoculated sentinels.

The Study

In 2005, we challenged reindeer fawns from a farm in Alaska, USA, where CWD had never been reported, by intracranial inoculation (*7*) with pooled brain material from CWD-affected elk from South Dakota (CWD^{elk}), CWD-affected mule deer from Wyoming (CWD^{md}), or CWD from white-tailed deer from Wisconsin combined with brain material from experimentally challenged white-tailed deer (CWD^{wtd}) (Table 1; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/12/16-0635-Techapp1.pdf). Additional uninoculated fawns served as negative controls, controls for indirect transmission, and controls for direct transmission (Table 1; online Technical Appendix). We determined the prion protein gene (*PRNP*) genotype of each fawn (online Technical Appendix), and we tried to ensure that each *PRNP* genotype was present in each group (Table 2, http://wwwnc.cdc.gov/EID/article/22/12/16-0635-T1.htm). Control reindeer were housed in the same barn as inoculated reindeer but in separate pens that prevented direct physical contact (i.e., nose-to-nose) between control and inoculated animals (online Technical Appendix Figure 1). Indirect and direct contact control groups were formed 25 months after intracranially challenged reindeer were inoculated (online Technical Appendix Figure 1, panel B).

Clinical signs consistent with CWD were first observed 20.9 months after inoculation (Table 2). Common clinical features included found dead without clinical signs noted, loss of body condition, recumbency, and lethargy (Table 2; online Technical Appendix).

At death, a full necropsy was performed on all reindeer. Two sets of tissue samples were collected: 1 set was fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 μm for microscopy examination after hematoxylin and eosin staining or immunohistochemical staining using primary antibody F99/96.7.1 (online Technical Appendix). A second set of tissues was frozen, and selected tissues were used for immunodetection of scrapie prion protein (PrP^{Sc}) by Western blot (brain tissue only) as described previously (*7*) but with some modifications, or an ELISA (brainstem and/or retropharyngeal lymph node) using a commercial kit (IDEXX HerdChek BSE-Scrapie Antigen ELISA; IDEXX, Westbrook, ME, USA) according to the manufacturers' instructions (online Technical Appendix).

In the intracranially inoculated groups, when intercurrent deaths were excluded, reindeer with the NN138 polymorphism (reindeer nos. 2, 6, and 12) had the shortest survival times in each group (Table 2). Different inocula did not produce significantly different survival times (log-rank

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[1]Deceased.

Table 1. Animal data for reindeer (*Rangifer tarandus tarandus*) in a study of transmission of CWD*

Group no./animal no.	Genotype codon 002	129	138	169	176	Infectivity source	Exposure route
1							
1	MV	SG	NS	MV	NN	CWD^{wtd}	Intracranial
2	VV	GG	NN	VV	NN	CWD^{wtd}	Intracranial
3	VV	GG	NS	VV	ND	CWD^{wtd}	Intracranial
4	VV	GG	NS	VV	NN	CWD^{wtd}	Intracranial
5	MV	SG	SS	MV	ND	CWD^{wtd}	Intracranial
2							
6	VV	GG	NN	VV	NN	CWD^{elk}	Intracranial
7	MV	SG	NS	MV	NN	CWD^{elk}	Intracranial
8	VV	GG	NS	VV	NN	CWD^{elk}	Intracranial
9	VV	GG	NS	VV	ND	CWD^{elk}	Intracranial
10	NA	SG	SS	MV	NN	CWD^{elk}	Intracranial
3							
11	MV	SG	NS	MV	NN	CWD^{md}	Intracranial
12	VV	GG	NN	VV	NN	CWD^{md}	Intracranial
13	VV	GG	SS	VV	DD	CWD^{md}	Intracranial
14	MV	SG	SS	MV	NN	CWD^{md}	Intracranial
15	VV	GG	NS	VV	ND	CWD^{md}	Intracranial
4 direct							
16	VV	GG	NN	VV	NN	Horizontal (CWD^{wtd})	Cohoused with group 1
17	VV	GG	NN	VV	NN	Horizontal (CWD^{wtd})	Cohoused with group 1
18	VV	GG	NN	VV	NN	Horizontal (CWD^{wtd})	Cohoused with group 1
19	NA	SG	NS	MV	NN	Horizontal (CWD^{wtd})	Cohoused with group 1
4 indirect							
20	MM	SS	SS	MM	NN	Horizontal (CWD^{md})	Housed adjacent to group 3
21	VV	GG	NN	VV	NN	Horizontal (CWD^{md})	Housed adjacent to group 3
4 neg. controls							
22	VV	GG	NS	VV	NN	NA	NA
23	MV	SG	SS	MV	NN	NA	NA

*CWD, chronic wasting disease; D, aspartic acid; G, glycine; horizontal, horizontal transmission; M, methionine; md, mule deer (*Odocoileus hemionus*); N, asparagine; NA, not applicable; neg., negative; S, serine; V, valine; wtd, white-tailed deer (*Odocoileus virginianus*).

test, p = 0.0931), but we observed differences in the amount of vacuolation and PrP^{Sc} in the brain at the clinical stages of disease in CWD^{wtd}- and CWD^{elk}-inoculated reindeer, compared with CWD^{md}-inoculated reindeer (Table 2; online Technical Appendix). In the indirect contact animals, PrP^{Sc} was present in the brain but restricted to the dorsal motor nucleus of the vagus nerve and area postrema.

We observed different patterns of PrP^{Sc} deposition in the brain (Figure 1, panels A–D; online Technical Appendix), the most striking of which was dominated by aggregated deposits of various sizes, including plaque-like deposits (Figure 1, panels A,B). This pattern was seen in reindeer with the NS138 NN176 (no. 8, CWD^{elk}; no. 13, CWD^{md}) or SS138 DD176 (no. 4, CWD^{wtd}) genotypes. With regard to immunoreactivity in the retina (Figure 1, panels E, F; online Technical Appendix), in 2 of 3 reindeer with aggregated deposits in the brain (nos. 8 and 13), aggregated immunoreactivity also was observed in the inner plexiform layer of the retina (Figure 1, panel f).

Reindeer that were negative by immunohistochemical analysis in brain also were negative by Western blot and ELISA. Different Western blot migration patterns were observed in PrP^{Sc}-positive animals (Figure 2), but we found no clear association between migration pattern and challenge group or *PRNP* genotype.

PrP^{Sc} was widespread in lymphoid tissues from most reindeer (Table 2; online Technical Appendix). Reindeer with the NS138 genotype had a significantly lower average percentage of lymphoid follicles positive than did reindeer with NN138 (analysis of variance, p = 0.003) or SS138 (p = 0.003) deer. Excluding intercurrent deaths, PrP^{Sc} was detected in all 4 CWD^{wtd}-challenged reindeer, all 5 CWD^{elk}-challenged reindeer, all 4 CWD^{md}-challenged reindeer, both indirect contact reindeer, and 2 of 4 direct contact reindeer (Table 2).

Conclusions

Potential sources of infectivity for direct contact animals include urine, feces, and saliva from their CWD^{wtd}-challenged pen-mates, as has been shown for CWD-affected white-tailed deer (*6*,*8*,*9*). Pinpointing the source of infectivity in the indirect contact group is more difficult. Infectious prions can travel at least 30 m in airborne particulate (*10*), but because the negative control reindeer in the pen adjacent to the indirect contact reindeer did not become positive, a more direct route of transmission is likely in this case. Penning, feeding, and watering protocols were designed to prevent exposure of negative control and indirect contact reindeer to potential infectivity on feed and water buckets, bedding, or fencing (*6*,*11*). However, reindeer

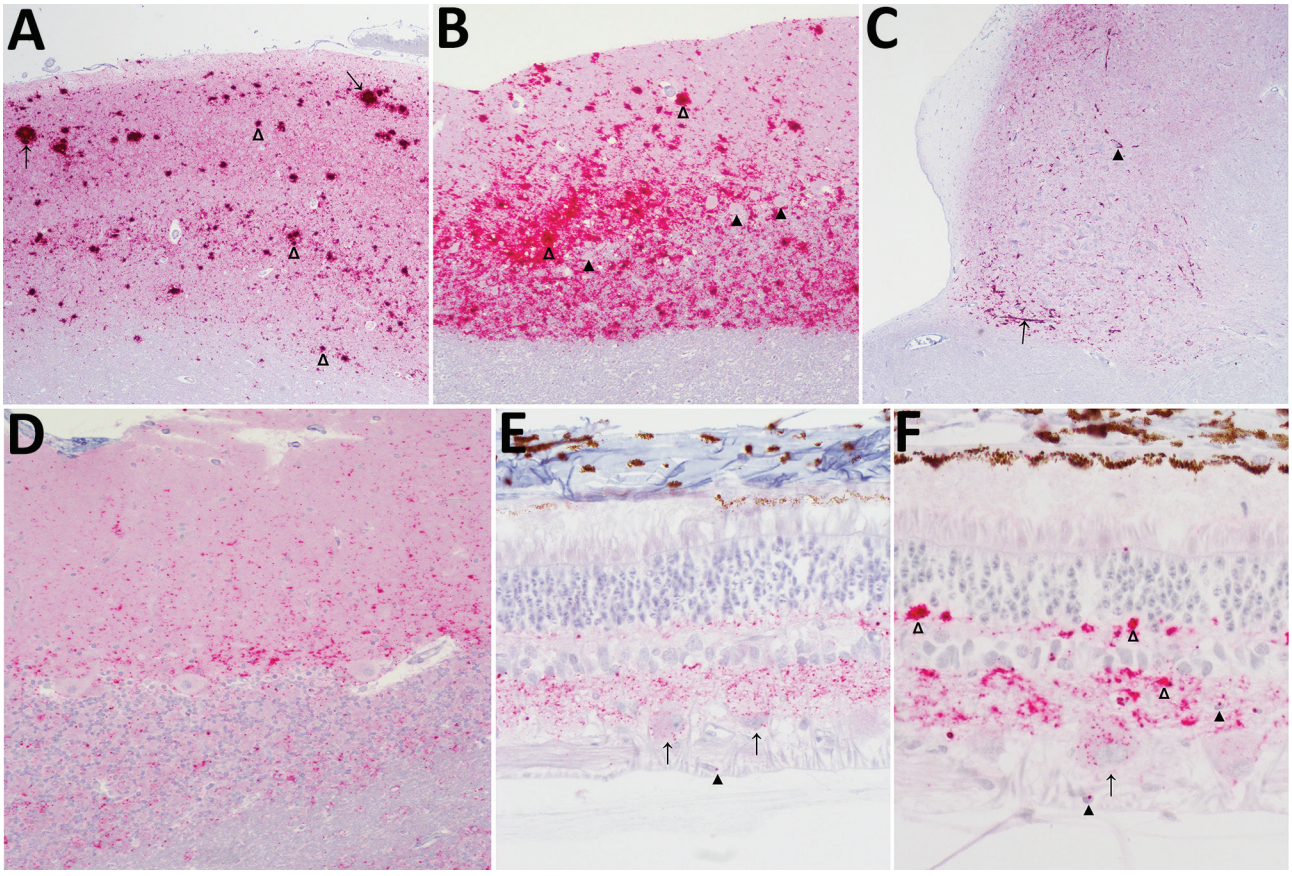

Figure 1. Immunohistochemical analysis for the prion protein showing scrapie prion protein (PrP^{Sc}) deposits in brains (A–D) and retinas (E, F) from reindeer (*Rangifer tarandus tarandus*) with chronic wasting disease. PrP^{Sc} immunodetection using the monoclonal antibody F99/97.6.1. A) Neocortex, showing prominent aggregated (open arrowheads) and plaque-like (arrows) deposits in reindeer no. 4. Original magnification ×5. B) Cerebellum, showing particulate immunoreactivity and aggregated deposits (open arrowheads) in reindeer no. 4. Note absence of intraneuronal immunoreactivity in Purkinje cells (solid arrowheads). Original magnification ×10. C) Brainstem at the level of the obex, showing prominent linear (arrow) and perineuronal (solid arrowhead) immunoreactivity in the dorsal motor nucleus of the vagus nerve in reindeer no. 21. Original magnification ×5. D) Cerebellum, punctate immunoreactivity in the molecular and granular layers and white matter in reindeer no. 12. Original magnification ×5. E) Intraneuronal immunoreactivity in retinal ganglion cells (arrows), punctate deposits in the inner and outer plexiform layers, scattered intramicroglial deposits (solid arrowheads) in reindeer no. 12. Original magnification ×40. F) Particulate to coalescing deposits in the inner and outer plexiform layers (open arrowheads), intraneuronal immunoreactivity in retinal ganglion cells (arrows), and scattered intramicroglial deposits (solid arrowheads) in reindeer no. 13. Original magnification ×40.

might have had access to bedding from adjacent pens that had spread into the central alleyway.

During the 5-year course of this study, reindeer were moved between pens several times to maintain an optimal number of animals per pen (online Technical Appendix Figure 1). Prolonged persistence of prion infectivity in the natural environment has been documented for both CWD (2 years [*5*]) and scrapie (up to 16 years [*12*]). In addition, thorough cleaning and disinfection might not be sufficient to remove all infectivity from the environment, leading to persistence of infectivity under experimental housing conditions (*13*).

In reindeer challenged orally with the agent of CWD, the SS138 genotype (serine/serine at *PRNP* codon 138) has been associated with susceptibility to disease and the NS138 (asparagine/serine) genotype with resistance (*1*). In the study we report, disease developed in reindeer with the NS138 genotype after intracranial inoculation, although the extent of lymphoreticular system involvement was significantly lower than in NN138 and SS138 reindeer. The potential association of the NN138 polymorphism with shorter survival times is interesting. However, as with all potential genotype versus phenotype interactions, care should be taken not to over-interpret these results given the small group sizes and the large number of *PRNP* genotype groups in this study.

Our results demonstrate that reindeer are susceptible to the agent of CWD from white-tailed deer, mule deer, and elk sources after intracranial inoculation. Furthermore, naive reindeer are susceptible to the agent of CWD after

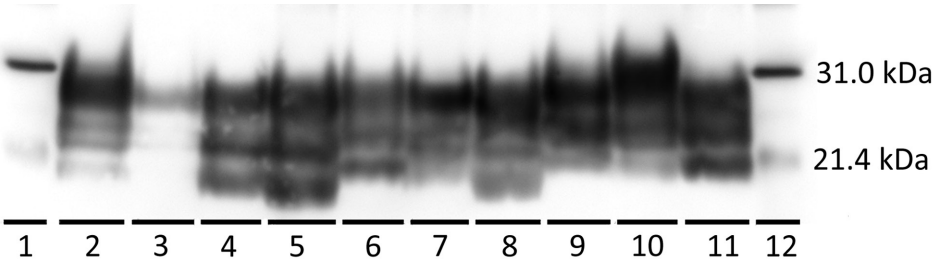

Figure 2. Western blot characterization of the inocula used to inoculate reindeer and brainstem samples from representative reindeer from each experimental group in study of chronic wasting disease transmission. Scrapie prion protein (PrP^{Sc}) immunodetection using the monoclonal antibody 6H4. Positive Western blot results demonstrate a 3-band pattern (diglycosylated, highest; monoglycosylated, middle; and nonglycosylated, lowest) that is characteristic of prion diseases. Lanes: 1, biotinylated protein marker; 2 and 3, indirect contact reindeer (animals no. 20 and 21, respectively); 4 and 5, reindeer inoculated intracranially with CWD^{md} (animals no. 15 and 12 respectively); 6, CWD^{md} inoculum; 7, direct contact reindeer (no. 7, cohoused with CWD^{wtd}-inoculated reindeer); 8, reindeer (no. 5) inoculated intracranially with CWD^{wtd}; 9, CWD^{wtd} inoculum; 10, reindeer (no. 10) inoculated intracranially with CWD^{elk}; 11, CWD^{elk} inoculum; 12, marker. CWD, chronic wasting disease; CWD^{elk}, CWD-affected elk; CWD^{md}, CWD-affected mule deer; CWD^{wtd}, CWD-affected white-tailed deer combined with brain material from experimentally challenged white-tailed deer.

direct and indirect exposure to CWD-infected reindeer, suggesting a high potential for horizontal transmission of CWD within and between farmed and free-ranging reindeer (and caribou) populations.

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Technical Appendix

Materials and Methods

Ethics and Safety Statement

This experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, DC, USA) and the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, Champaign, IL, USA). The protocol was approved by the Institutional Animal Care and Use Committee at the National Animal Disease Center (NADC protocol no. 3669).

Inoculation Procedure

At 6 months of age, reindeer fawns received an intracranial inoculation of 1 mL 10% brain homogenate, as described previously for sheep (*1*).

Inoculum

The inocula were prepared from pooled brain material from chronic wasting disease–affected elk from South Dakota, USA (CWD^{elk}), and mule deer from Wyoming, USA (CWD^{md}), as described previously (*2*). The inoculum from CWD-infected white-tailed deer (CWD^{wtd}) was prepared from pooled brainstems from 3 white-tailed deer; 1 deer had been inoculated intracranially with the CWD^{elk} inoculum, the second deer with the CWD^{md} inoculum, and the third deer with pooled brain material from CWD-affected white-tailed deer from Wisconsin (*2*). All deer from which the inocula were prepared were positive for the pathogenic form of the scrapie prion protein (PrP^{Sc}) by immunohistochemistry (IHC), and the pooled inocula were positive by Western blot. The brain material for the inocula was homogenized to 10% (w/v) in phosphate-buffered saline; pH 6.15), and gentamicin was added (to 100 μg/mL).

Animals

Twenty-three 3-month-old reindeer fawns were obtained from a farm in Alaska, USA, that had never had a reported case of CWD. Reindeer were used in preference to caribou because they are semidomesticated and so more appropriate for experimental studies. The fawns were divided into 4 groups: group 1 (n = 5) was inoculated with CWD^{wtd}; group 2 (n = 5) was inoculated with CWD^{elk}; group 3 (n = 5) was inoculated with CWD^{md}; and group 4 (n = 8) remained as negative controls (n = 2) or were placed in direct (n = 4) or indirect (n = 2) contact groups.

Genotyping

Genotype analysis was conducted on nucleic acid extracted from blood samples from live animals, as described previously (*3*). The nucleotide numbering for the prion protein gene (*PRNP*) sequence is based on the GenBank *Rangifer tarandus tarandus* sequence (accession no. AY639093.1).

Animal Housing and Pen-to-Pen Movement

All reindeer were housed in an animal biosafety level 2 containment facility at the National Animal Disease Center, US Department of Agriculture (Ames, IA, USA), and were monitored twice daily during the experiment. Control reindeer were housed in the same barn as inoculated reindeer but in separate pens that prevented direct physical contact (i.e., nose-to-nose) between control and inoculated animals (Technical Appendix Figure, panel A).

Twenty-five months after intracranially challenged reindeer were inoculated, 4 control reindeer (reindeer #16, #17, #18, #19) were moved into the same pen as CWD^{wtd}-inoculated reindeer (Technical Appendix Figure, panel B, pen 5+7) to form the direct contact control group (Table 1, group 4 direct). At the same time, 2 control reindeer (#20, #21) were moved into pen 6, which was adjacent to pen 4 which housed the CWD^{md}-inoculated reindeer (Technical Appendix Figure, panel B, pen 4, pen 6) to form the indirect contact control group. Solid partitions between pen 4 and pen 6 prevented physical contact between the reindeer in these 2 pens; however, reindeer in both pens could reach through the front of the pen to access a central alleyway that could contain bedding or water from adjacent pens generated during daily cleaning. The 2 control reindeer (#22, #23) in pen 8 formed the negative control group (Technical Appendix Figure, panel B, pen 8).

Forty-four months later, CWD^{md}-inoculated reindeer #15 was moved from pen 4 to join reindeer #13 in pen 2 (Technical Appendix Figure, panel C). Pen 4 was cleaned by manual sweeping to remove bedding, etc., followed by pressure washing with water, followed by decontamination with 20,000 ppm sodium hypochlorite for a 1h contact time. Group 4 indirect contact reindeer were moved from pen 6 to pen 4. Pen 6 was cleaned and decontaminated, and then group 4 control reindeer were moved from pen 8 to pen 6. Reindeer remained in these pens until the end of the study.

Survival Times

Survival times for intracranially challenged reindeer are expressed as months postinoculation (MPI) and are calculated from the day the reindeer were inoculated. Survival times for direct and indirect contact reindeer are expressed as months postchallenge and are calculated from the date that direct contact control reindeer were mixed with CWD^{wtd}-inoculated reindeer.

Immunohistochemistry

All paraffin-embedded tissues were stained by an automated IHC method for detection of PrP^{Sc} as described previously (*3*) using an automated immunostainer (Ventana Medical System Inc, Tucson, AZ, USA). The primary antibody was F99/97.6.1 (*4*), used at a concentration of 10 µg/mL, and incubation was carried out at 37°C for 32 min.

ELISA

Frozen brainstem (at the level of the obex) and/or retropharyngeal lymph node were used for detection of PrP^{Sc} using the IDEXX HerdChek BSE-Scrapie Antigen ELISA (IDEXX, Westbrook, ME, USA). For animals that were negative by IHC, brainstem and retropharyngeal lymph node samples were processed as per kit instructions. For animals that were positive by IHC, brainstem samples were homogenized in phosphate-buffered saline to enable testing of the same sample by both ELISA and Western blot.

ELISA was performed according to the kit instructions (short protocol) with the following modifications: capture plate incubation was performed for 1.5 h; conjugate incubation was performed for 1 h. Samples were run as singles with kit-provided negative and positive controls included in each run. Small ruminant brain conjugate was used for all samples.

Absorbance was measured using a SpectraMax 190 plate reader at 450 nm with a reference wavelength of 620 nm.

Western Blot

Frozen brain tissues were used for immunodetection of PrP^{Sc} by Western blot using a previously published protocol (*3*) with the following modifications: samples from the brainstem were homogenized at a final concentration of 10% or 20% (w/v) in 1X Dulbecco's Phosphate Buffered Saline (free of calcium or magnesium, Mediatech Inc., Manassas, VA, USA) using a tissue homogenizer (Biotech Mini-BeadBeater-16, BioSpec Products, Bartlesville, OK, USA) and imaged using a multimode scanner (G:BOX Chemi XT4, Syngene, Frederick, MD, USA). Immunodetection was conducted using mAb 6H4 (Prionics AG, Schlieren-Zürich, Switzerland) at a 1:10 000 dilution (0.1 μg/mL).

Results

Clinical Presentation

Clinical signs included loss of body condition (n = 5), recumbency (n = 4), and lethargy (n = 2). Seven reindeer were found dead without clinical signs noted. Seizures developed in 1 CWD^{wtd}-inoculated reindeer (#4), and it was euthanized. Bloat that was not responsive to treatment developed in a reindeer (#21) from the indirect contact group, and it was euthanized. There were 2 intercurrent deaths (pneumonia) at 2.6 (#1) and 13.7 (#11) MPI.

Vacuolation and PrP^{Sc} Distribution in Central Nervous System Tissues

Neither neuropil nor neuronal vacuolation was seen in direct or indirect contact animals. PrP^{Sc} was not detected in the brains from any direct contact animals, including the 2 reindeer that had PrP^{Sc} in non–central nervous system (CNS) tissues (#17 and #18).

All 4 CWD^{wtd}-inoculated reindeer had both CNS vacuolation and PrP^{Sc} accumulation at clinical stages (20.9–53.3 MPI), except for 1 intercurrent death (#1) at 2.6 MPI. Both vacuolation and PrP^{Sc} accumulation were seen in the brain of the CWD^{elk}-inoculated reindeer that was found dead at 24.7 MPI (#6). A second CWD^{elk}-inoculated reindeer was found dead at 36.4 MPI (#7) without microscopic evidence of spongiform encephalopathy. The remaining 3 CWD^{elk}-inoculated reindeer had widespread CNS vacuolation and PrP^{Sc} accumulation at death. In the CWD^{md}-inoculated group, widespread vacuolation and PrP^{Sc} accumulation were present in

reindeer that survived up to 30 months (n = 2, #12 and #13). In contrast, reindeer with survival times >30 months showed widespread PrP^{Sc} accumulation but minimal (#15, thalamus and frontal cortex only) or no (#14) vacuolation.

PrP^{Sc} was present in the brains of intracranially inoculated and indirect contact reindeer. The most striking pattern of PrP^{Sc} deposition in the brain was dominated by aggregated deposits of various sizes, including plaques, distributed throughout the neuroaxis (Figure 1, panels A,B). This pattern was seen in 3 reindeer (#4, #8, and #13) and was associated with the NS138 NN176 (n = 2, #8 CWD^{elk}, #13 CWD^{md}), or SS138 DD176 (#4 CWD^{wtd}) genotype. There was not a consistent association between aggregated PrP^{Sc} deposits and distribution or severity of vacuolation. A second pattern, comprising perineuronal and linear labeling with a restricted distribution, confined to the dorsal motor nucleus of the vagus (Figure 1, panel C), midbrain, hypothalamus, and midline thalamic nuclei, was observed in 4 reindeer of the NN138 NN176 (n = 3, #2, #6, and #21) or SS138 ND176 (#5) genotype. A third pattern of widespread punctate neuropil and intraneuronal labeling (Figure 1, panel D) was observed in 4 reindeer and was associated with the NS138 ND176 (n = 3, #3, #9, and #15) or NN138 NN176 (#12) genotype. The fourth pattern comprised neuropil labeling confined to the brainstem at the level of the obex (hypoglossal nucleus, dorsal motor nucleus of the vagus nerve, area postrema) and midbrain (trochlear nuclei) that was observed in 3 reindeer of the SS138 NN176 genotype (#10, #14, and #20).

All animals with PrP^{Sc} immunoreactivity in brainstem also had PrP^{Sc} in the retina (Table 2). In the optic fiber layer, PrP^{Sc} immunoreactivity was observed as mild punctate deposits and/or rare intramicroglial deposits (Figure 1, panel E). Retinal ganglion cells were negative in most reindeer, but intraneuronal immunolabeling of retinal ganglion cells (Figure 1, panels E,F) was observed in 4 reindeer (#3, #6, #12 [Figure 1, panel E], #13 [Figure 1, panel F]). The most commonly observed PrP^{Sc} immunolabeling pattern in the inner plexiform layer was mild to moderate punctate deposits with occasional intramicroglial deposits (Figure 1, panel E). In 2 reindeer (#8 and #13) moderate particulate to coalescing immunolabeling was observed in the inner plexiform layer (Figure 1, panel F); notably, both of these animals had aggregated and plaque-like PrP^{Sc} deposits in the brain. PrP^{Sc} in the outer plexiform layer was present as very mild to moderate granular labeling.

PrP^{Sc} Distribution in Non-CNS Tissues

The tissue distribution of PrP^{Sc} was similar for all intracranially inoculated reindeer (Table 2) except reindeer #7 (CWD^{elk}, survival time 36.4 MPI) that was IHC positive in retropharyngeal and popliteal lymph nodes only and reindeer #15 (CWD^{md}, survival time 43.5 MPI) that was IHC positive in retina, pituitary, and trigeminal ganglion only. The nasal turbinates, trachea, lung, biceps femoris muscle, triceps muscle, diaphragm, heart, tongue, salivary gland, esophagus, reticulum, duodenum, thyroid, pancreas, urinary bladder, sciatic nerve, skin, and antler velvet were negative in all samples examined by IHC.

The retropharyngeal lymph node, pharyngeal tonsil, palatine tonsil, mesenteric lymph node, spleen, prescapular lymph node and popliteal lymph node were IHC positive in the majority (55–67%) of reindeer. The gut-associated lymphoid tissue of the ileum (Peyer's patches) and recto-anal junction (RAMALT) were IHC positive in 29.4% and 61.9% of reindeer, respectively. Twelve reindeer that had PrP^{Sc} in the RAMALT were overall IHC positive, and 2 reindeer (both direct contact group) were negative in the RAMALT and overall negative (Table 2). Samples of RAMALT from the remaining 5 reindeer were IHC negative, but PrP^{Sc} was demonstrated in other tissues (Table 2).

ELISA

Reindeer that were IHC negative in brain also were negative by Western blot and ELISA.

Western Blot

The nonglycosylated (lowest) band from the CWD^{md}, CWD^{elk}, and CWD^{wtd} inocula migrated at ≈21.4 kDa (Figure 2). In some instances, the migration patterns of the nonglycosylated band of challenged reindeer varied relative to the source inoculum (Figure 2). There was not a clear association between a higher or lower position of the nonglycosylated band and either challenge group or *PRNP* genotype.

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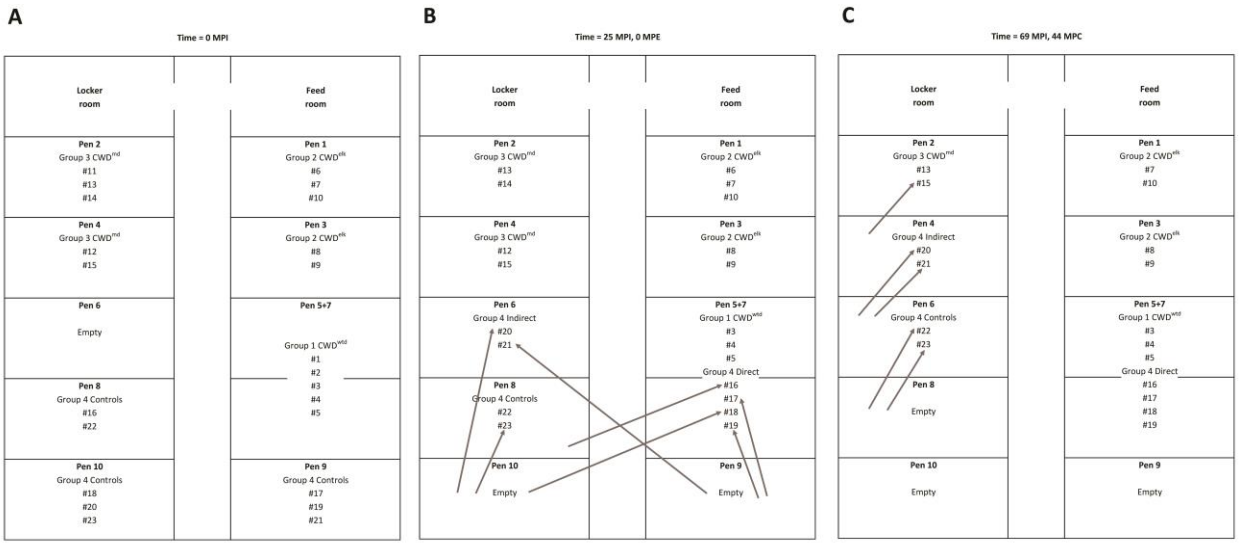

Technical Appendix Figure. Animal pen plan and animal movements. A) Pen locations at the beginning of the study (0 MPI). B) First round of animal movements (25 MPI, 0 MPC). C) Second round of animal movements (69 MPI, 44 MPC). CWD, chronic wasting disease; CWD^{md}, CWD from mule deer; CWD^{elk}, CWD from elk, CWD^{wtd}, CWD from white-tailed deer (see also Table 1); MPC, months postchallenge (direct and indirect control reindeer); MPI, months postinoculation (intracerebrally inoculated reindeer). Animal numbers (e.g., #1) refer to individual reindeer as in Tables 1, 2. Arrows: the base of the arrow indicates the pen the animal was in; the arrow head closest to the animal number indicates the pen the animal was moved to. For example: reindeer #21 began in pen 9 (A). At 25 MPC, reindeer #21 was moved from pen 9 to pen 6 (B). At 44 MPE, reindeer #21 was moved from pen 6 to pen 4 (C), where it remained for the rest of the study.